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Identification of phytochemicals in hydro alcohol extract of annona muricata fruit using GC-MS analysis

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Abstract

Investigation of phytochemicals present in hydroalcoholic fruit extract of *Annona muricata* using Gas chromatography and Mass spectroscopy (GC-MS) was done by as per standard protocol. The GC-MS analysis revealed the presence of various compounds like 2-Furancarboxaldehyde, 5-Methyl, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, Pentanoic acid, 4-oxo- Levulinic acid, 1,3,5-Triazine-2,4,6-triamine Melamine, dl-Glyceraldehyde dimer, Heptanoic acid, 6-oxo- 6-Oxoheptanoic acid, Tetradecanoic acid, Myristic acid, n-Tetradecanoic acid, 9,12,15-Octadecatrienoic acid, (Z,Z,Z), Octadecanoic acid, Stearic acid in hydroalcoholic fruit extract *Annona muricata*. These findings suggest that the fruit of *Annona muricata* contains variable and valuable bioactive compounds which has potential role in treating various disease condition.

Keywords: Gas chromatography and Mass spectroscopy, *Annona muricata* fruit, Hydroalcohol extract

Introduction

Advancement in analytical technique has been extensively utilized for identification, structural determination and separation of bioactive compounds in a mixture by utilizing NMR, GCMS and TLC (1). *Annona muricata* fruit possess lot of secondary metabolite, vitamins and mineral with variable valuable biological activity. Hence, we decided to carryout phytochemical screening in hydroalcohol fruit extract of *Annona muricata* using GCMS (1).

Annona genus belongs to Annonaceae family classified under flowering plant. Species is *muricata* Common name: Graviola, Soursop and guanabana. *Annona muricata* is widely distributed in tropical region in Southeast Asia such as India, China and in Central and South America (2). It's a tropical plant known for its edible fruit which has traditional medicinal values. *Annona muricata* has been quite useful in the treatment of diarrhea, intestinal parasites, liver, heart disease, Hypertension, Diabetes and Cancer (3, 4, 5, 6, 7). Moreover, they are also used for pest control (8). *Annona muricata* contains more than 200 bioactive compounds.

Collection of plant and fruit material

The collected plant and fruit was authenticated (VS001) by Dr.S.John Britto, The Director, The Rapiant Herbarium and Centre for Molecular Systematics, St. Joseph's College (Campus) Trichy-Tamilnadu.

Plant materials and preparation of hydroalcohol fruit extract

The *Annona muricata* fruit was collected from Trivandrum, Kerala, India. The collected fruits were weighed, washed, finely sliced and completely dried in a hot-air oven at 37°C. The dried materials was ground to make a fine powder and used for extraction. Two grams (2g) of the powder fruit was macerated with methanol (70%) at room temperature for 3 days. The supernatant was transferred into china dish kept in boiling water bath at 45 °C. A semi solid extract was obtained after complete elimination of alcohol. The extract was stored in refrigerator (-20°C) until used. The extract contains both polar and non-polar phytocomponents. This extract will be used for further studies.

GC-MS analysis

GC 500 Perkin Elmer system has been utilized for analysis of Hydroalcohol fruit *annona muricata* extract which include an autosampler and gas chromatograph interfaced to a mass spectrometer instrument.

GC Program

- Elite column -1 fused with silica capillary column spec (30 X 0.25mm Internal Diameter X1μMdf, comprised of 100% Dimethyl polydioxane)

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- Carrier gas used is Helium (99.99%) with flow rate 1ml/min, split ratio 10:1
- Detector- Mass detector turbo mass gold-perkin Elmer
- TurboMass Ver 5.2.0 software utilized
- Sample injected volume 0.5 µl

Oven Temperature Program

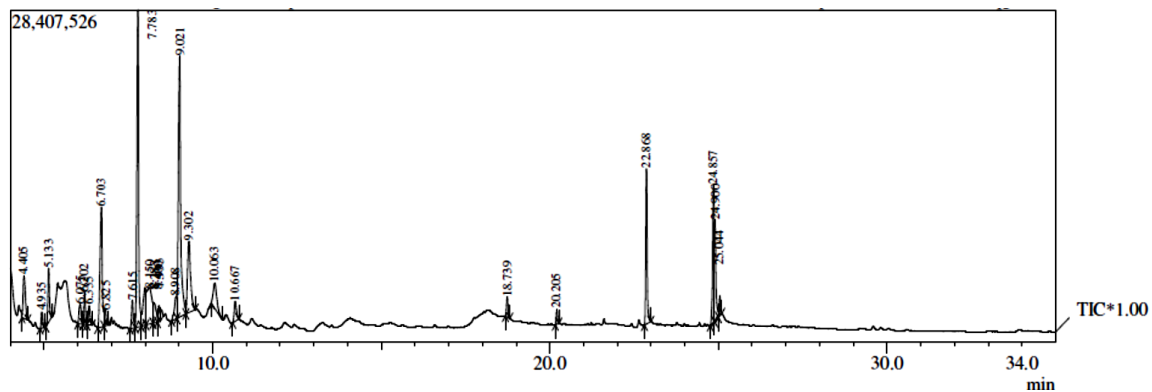
- 110° for 2min hold
- Increase in 10°/min until it reach 200° -no hold
- Increase in 5°/min up to 280° with 9min hold
- Injector temperature 250° maintained
- Total GC running time is 36min

MS Programme

- Library used is NIST
- Electron temperature 70eV
- Mass scan(m/z) 45-450Da
- Ion source temperature maintained at 280 °C
- Scan interval 0.5 sec

Results and Discussion

GCMS Chromatogram of Hydralcoholic fruit extract of *Annona Muricata* Figure 1



GCMS is a specific test positively identifies the actual presence of a particular substance in a given sample.

Most of the active secondary metabolite present in hydroalcohol fruit extract of *Annona muricata* are in agreement with earlier studies performed on *Annona muricata* stem, leaf extract. The hydroalcohol fruit extract of *Annona muricata* chromatogram (Figure 1, Table 1) showed 25 peaks with retention time ranging from 4.405-25.044. The peak at retention time 4.4055 is having a small peak area 2.93% for 2-Cyclopentene-1-one,2-hydroxy. The last peak area observed is 18.73% with retention value 9.021 for 5-(Hydroxy Methyl)-2-Furaldehyde followed by peak area 16.88%, 8.38%, 6.89%, 6.64% and 6.53% with retention time of 7.783, 6.703, 24.857, 9.302 and 22.868 corresponds to 4H-Pyran-4-one, 2, 3-

dihydro-3, 5-dihydroxy-6-methyl, 1, 3, 5-Triazine-2, 4, 6-triamine \$\$ Melamine \$, 9-Octadecenoic acid, (E)- \$\$ trans-.delta.(sup 9)-Octadecenoic acid, 2,3-DIHYDROXYPROPYL ACETATE and 1-(+)-Ascorbic acid 2,6-dihexadecanoate. Biological value of compounds is listed (Table2) which is based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA

Antidiabetic, Antiproliferative, Anticancer and inhibitory effect on bacteria and fungus, multiple protective effects of *Annona Muricata* is due to active metabolites present in plant and our GCMS analysis performed on hydroalcohol extract of *Annona muricata* shows the major bioactive metabolite present in our extract. Further in-depth studies are required.

Table 1: Shows the components identified in hydroalcohol fruit extract of *Annona muricata* (GC MS study)

Peak#	R.Time	Area%	Height%	Molecular formula	Molecular weight	Name
1	4.405	2.93	2.62	C5H6O2	98	2-Cyclopenten-1-one, 2-hydroxy- \$
2	4.935	0.7	1	C6H6O2	110	2-FURANCARBOXALDEHYDE, 5-METHYL-
3	5.133	2.59	3.35	C6H8O4	144	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
4	6.075	1.31	1.21	C5H6O3	114	1,3-Dioxol-2-one,4,5-dimethyl
5	6.202	1.23	1.91	C5H8O3	116	Pentanoic acid, 4-oxo- \$\$ Levulinic acid
6	6.335	1.1	1.18	C6H8O3	128	2,5-ANHYDRO-1,6-DIDEOXYHEXO-3,4-DIULOSE
7	6.703	8.38	7.4	C3H6N6	126	1,3,5-Triazine-2,4,6-triamine \$\$ Melamine \$
8	6.825	1.04	1	C5H10N2O3	146	Glycylsarcosine \$\$ Glycine, N-glycyl-N-methyl-
9	7.615	1.52	1.75	C6H8O4	144	2-ACETYL-2-HYDROXY- GAMMA.-BUTYROLACTONE
10	7.783	16.88	19.59	C6H8O4	144	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
11	7.993	3.29	2.34	C4H4N2O5	160	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5,5-dihydroxy-
12	8.15	5.81	2.14	C6H12O6	180	dl-Glyceraldehyde dimer
13	8.283	1.61	1.12	C5H6O3	114	(S)-5-Hydroxymethyl-2[5H]-furanone \$\$
14	8.4	0.82	1.01	C6H6O4	142	2-METHYL-2H-PYRAN-3,4,5(6H)-TRIONE \$\$
15	8.908	1.72	1.35	C10H18O2	170	2(3H)-FURANONE, 5-HEXYLDIHYDRO- \$\$, GAMMA.
16	9.021	18.73	16.15	C6H6O3	126	5-(HYDROXYMETHYL)-2-FURALDEHYDE \$\$
17	9.302	6.64	4.4	C5H10O4	134	2,3-DIHYDROXYPROPYL ACETATE \$
18	10.063	2.72	1.56	C7H12O3	144	Heptanoic acid, 6-oxo- \$\$ 6-Oxoheptanoic acid # \$\$
19	10.667	1.37	1.24	C5H13N3	115	1-Amino-4-methylpiperazine \$\$ 1-Piperazinamine, 4-methyl-
20	18.739	0.91	1.26	C11H22BN	179	N-PROPYL-9-BORABICYCLO[3.3.1]NONAN-9-AMINE
21	20.205	0.57	0.91	C14H28O2	228	Tetradecanoic acid \$\$ Myristic acid \$ n-Tetradecanoic acid
22	22.868	6.53	9.58	C38H68O8	652	1-(+)-Ascorbic acid 2,6-dihexadecanoate
23	24.857	6.89	8.46	C18H34O2	282	9-Octadecenoic acid, (E)- \$\$ trans-.delta.(sup 9)-Octadecenoic acid
24	24.9	3.92	6.24	C18H30O2	278	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
25	25.044	0.81	1.24	C18H36O2	284	Octadecanoic acid \$\$ Stearic acid \$\$

Table 2: Activity of phyto-components identified in the hydroalcohol fruit extracts of the *Annona muricata* by GC-MS

Retention Time	Name of the compound	Biological Value
4.935	2-Furancarboxaldehyde, 5-Methyl-	Antimicrobial, Preservative, Antioxidant
5.133	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	Antioxidant
6.202	Pentanoic acid, 4-oxo- \$\$ Levulinic acid	Potential biofuels can be prepared. Also used in cigarettes to increase nicotine delivery in smoke and binding of nicotine to neural receptors
6.703	1,3,5-Triazine-2,4,6-triamine \$\$ Melamine \$	Allergenic compound. Irritation-Eye, Nose, Throat, Skin (OSHA, United State Dept. of Labor).
8.15	dl-Glyceraldehyde dimer	Sugar moiety and Preservative
10.063	Heptanoic acid, 6-oxo- \$\$ 6-Oxoheptanoic acid # \$\$	Antiviral
20.205	Tetradecanoic acid \$\$ Myristic acid \$\$ n-Tetradecanoic acid	Nematicide, Hypocholesterolemia, Antioxidant, Cancer prevention, Lubricant. It is used in cosmetic and topical medicinal preparations where good absorption through the skin is desired.
24.9	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	Anti-inflammatory, Cancer preventive, Hepatoprotective, Nematicide, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Acidifier, Inhibitor of arachidonic acid, Increase Aromatic amino acid decarboxylase activity
25.044	Octadecanoic acid \$\$ Stearic acid \$\$	Antioxidant, Acidifier, Inhibitor of arachidonic acid, Inhibit production of uric acid, Urine acidifier 5- α reductase inhibitor, cosmetic, lubricant, surfactant & softening agent, perfumery, flavor agent

**Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database]

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